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Commentary

Interleukin-1β converting enzyme (caspase-1) in intestinal inflammation

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Abstract

An imbalance of T helper cell type 1 (Th1) versus type 2 (Th2) polarization in favor of Th1 cell subsets appears to be a key pathogenic mechanism in chronic inflammatory bowel disease (IBD), in particular in Crohn's disease. The interferon γ-inducing factor interleukin (IL)-18 acts in strong synergism with the Th1 polarizing cytokine IL-12. Recent studies provide evidence for the participation of IL-18 in the pathogenesis of IBD: IL-18 expression is increased in inflamed lesions of Crohn's disease patients and neutralization of IL-18 in different models of experimental colitis resulted in a dramatic amelioration of disease severity. IL-18 and IL-1β are cleaved and thereby activated by the interleukin-1β converting enzyme (ICE). Activation of ICE also occurs during different types of infectious colitis, and ICE expression and subsequent release of IL-1β and IL-18 significantly contribute to intestinal inflammation. *ICE* knockout mice as well as mice treated with the ICE inhibitor pralnacasan are protected against experimental mucosal inflammation. Thus, inhibition of ICE represents an intriguing new target that requires further investigation in animal models.

Keywords: Inflammatory bowel disease; Interleukin-1β converting enzyme; IL-18; IL-1β; Experimental colitis

1. Introduction

Human IBD is a chronic, relapsing, and remitting inflammatory condition of unknown origin that afflicts individuals of both sexes throughout life. Studies in humans with IBD suggest that genetic and environmental factors contribute to the pathogenesis of these disorders [1]. Studies in experimental models of mucosal inflammation provide profound insight into the understanding of pathologies of mucosal immunity, thereby guiding investigators towards new innovative therapeutic strategies for IBD.

Several approaches focusing on the inhibition of the pro-inflammatory Th1 response, such as treatment with a chimeric antibody against TNFα in patients with Crohn's disease [2] or administration of antibodies against IL-12 in experimental mucosal inflammation, have been shown to be dramatically effective [3,4]. Studies from the recent literature suggest that inhibition of the IFNγ-inducing factor IL-18, which strongly synergizes with IL-12, might be of therapeutic significance as well. IL-18 and IL-1β require cleavage by the ICE, also named caspase-1 [5], to become active mediators. The availability of ICE inhibitors offers a strong pharmacological tool for *in vivo* evaluation. The present review will focus on ICE, the biological activities of IL-18, the relevance of IL-18 neutralization during intestinal inflammation, and, hence, the possible significance of ICE inhibition.

2. ICE

2.1. Definition of ICE

ICE or caspase-1 is a member of a large family of intracellular cysteine proteases known as caspases. The term caspase stands for cysteine proteases cutting after aspartic acid [6]. The *ICE* gene codes for a 45 kDa inactive precursor protein that is constitutively expressed in many cell types. Whereas most caspases appear to be involved in the enzymatic pathways leading to apoptosis, ICE probably plays a lesser role in that function, and its predominant role is in the processing of IL-1β and IL-18. As with most caspases, the 45 kDa precursor of ICE requires two internal

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Abbreviations: ICE, interleukin-1β converting enzyme; Th1/2, T helper cell type 1/2; IL, interleukin; IBD, inflammatory bowel disease; IFNγ, interferon-γ; TNFα, tumor necrosis factor-α; DSS, dextran sulfate sodium; TNBS, trinitrobenzene sulfonic acid; IL-18BP, IL-18 binding protein; and hIL-18BPa, human IL-18BP isoform a.

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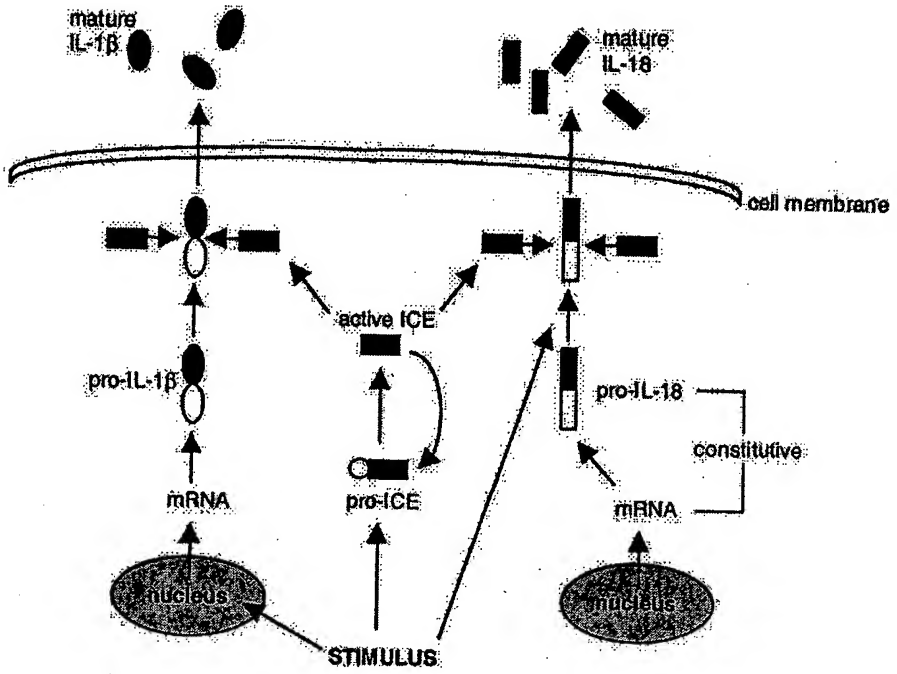

Fig. 1. Synthesis, ICE processing, and secretion of IL-1β and IL-18. A human monocyte is shown. After cell stimulation, mRNA for pro-IL-1β is induced and enters the cytosol. Pro-ICE is cleaved into active ICE by members of the caspase family including ICE itself. Pro-IL-1β is found diffusely in the cytosol and is cleaved by active ICE into mature IL-1β, which is secreted from the cell. Pro-IL-18 is expressed constitutively, as is the IL-18 mRNA. After stimulation of the monocyte, pro-IL-18 is cleaved by activated ICE and released.

cleavages before being enzymatically active as a heterodimer comprised of a 10 and 20 kDa chain. ICE itself contributes to autoprocessing of the ICE precursor by undergoing oligomerization with itself or other members of the caspase family, such as caspase-3 [7,8]. Each caspase appears to have limited substrate specificity: pro-IL-1β and pro-IL-18 have been identified as substrates for ICE. The activation of ICE and the subsequent activation of IL-1β and IL-18 are illustrated in Fig. 1 and will be discussed in more detail below.

2.2. Regulation of ICE

Activation of ICE is often associated with generalized cell activation and IL-1β and IL-18 production. Although endotoxin activates ICE as well as the synthesis of IL-1β, endogenous cytokines also possess this dual property. For example, the CD40 ligand, a member of the TNFα superfamily of ligands, activates ICE and leads to secretion of IL-1β from human endothelial and smooth muscle cells [9]. IFNγ also induces ICE expression, but not activation, in macrophages [10]. Not surprisingly, there is evidence that the regulation of ICE activity is affected by intracellular inhibitors. One such inhibitor is the serine proteinase inhibitor 9, which reduces ICE activity in cultured human smooth muscle cells [11]. Nitric oxide is a potent inhibitor of caspase activity through a mechanism that involves *S*-nitrosylation [12,13]. Accordingly, nitric oxide prevents the release of both IL-1β and IL-18 in the extracellular space [14].

2.3. Blockade of ICE in disease

The role of ICE has been characterized in several models of *in vivo* inflammation. ICE-deficient mice are resistant to lethal endotoxemia, but this is due to a failure to process pro-IL-18 and induce IFNγ rather than to inhibition of IL-1β [15,16]. In different models of acute pancreatitis, inhibition of ICE by the administration of an irreversible ICE inhibitor was shown to be protective [17,18]. Moreover, *ICE* knockout mice were found not to develop acute pancreatitis despite induction [19]. In addition, administration of an irreversible ICE inhibitor resulted in reduced inflammation in the mouse model of collagen-induced arthritis [20]. Furthermore, in melanoma studies, the number of hepatic metastases after intrasplenically injected mouse B16 melanoma cells was 84–95% lower in *ICE* knockout than in wild-type mice [21].

2.4. ICE in infectious intestinal inflammation

Three examples in the recent literature provide evidence that infection with distinct microorganisms and concurrent intestinal inflammation strongly depend on ICE activity. Enterobacteria of the genus *Shigella* are the causative agents of bacillary dysentery, manifested by painful abdominal cramps, fever, and characteristic blood and mucus in the stools [22]. Resident macrophages infected with *Shigella flexneri* undergo apoptosis [23,24] and release IL-1β, thereby initiating an inflammatory cascade [25–27]. Apoptosis induction as well as IL-1β maturation can be prevented by ICE inhibitors [28].

The protozoan parasite *Entamoeba histolytica*, which causes amoebic dysentery and amoebic liver abscesses, is one of the leading causes of death from parasitic diseases worldwide [29]. Intestinal inflammation and ulceration are the hallmarks of amoebic dysentery [30–32]. Amoebic cysteine proteases are mandatory as virulent factors, as cysteine proteinase-deficient amoeba failed to induce intestinal epithelial cell production of IL-1β and IL-18, accompanied by significantly reduced gut inflammation and damage to the intestinal permeability barrier [33].

Salmonella typhimurium invades host macrophages and can either induce rapid cell death or establish an intracellular niche within the phagocytic vacuole [34]. SipB, a protein translocated by *Salmonella* into the cytoplasm of macrophages, is required for the activation of ICE [35]. *ICE* knockout mice have an oral *Salmonella typhimurium* LD_{50} that is 100-fold higher than that of wild-type mice, accompanied by a decrease in apoptotic cells and decreased *Salmonella* dissemination [36,37]. These three examples point out that the activation of ICE and the subsequent release of IL-1β and IL-18 play a significant role in intestinal inflammation during infection.

2.5. ICE in IBD

A study on ICE in the context of IBD by McAlindon and colleagues [10] led to an important observation. Exposure of normal colonic macrophages to lipopolysaccharide induced only the production of the precursor of IL-1β, because the cells failed to activate ICE. In contrast, colonic macrophages from patients with IBD were able to activate ICE and hence release mature IL-1β in a manner similar to circulating monocytes. This is consistent with recent recruitment of IBD macrophages from the circulating monocyte population. Recent studies from our group examined the acute and chronic model of DSS-induced colitis in *ICE* knockout mice [38]. In particular, during chronic administration of DSS over 4 weeks, *ICE* knockout mice presented with an almost complete absence of colitis. This significant amelioration was accompanied by reduced cell activation in the draining mesenteric lymph nodes and a significant reduction of the pro-inflammatory cytokines IL-18, IFNγ, and IL-1β in the colon.

Several ICE inhibitors are available for experimental use *in vivo* or *in vitro*. Pralnacasan, an orally active inhibitor of human ICE, has been administered to healthy volunteers in phase I trials. The phase I and phase II clinical programs have confirmed that pralnacasan is well absorbed from oral solutions and tablet formulations and achieves plasma levels sufficient to inhibit production of IL-1β in an *ex vivo* assay. The preliminary safety profile from these studies in healthy volunteers and rheumatoid arthritis patients is excellent. Pralnacasan is currently in phase II trials in rheumatoid arthritis. No toxic side-effects have been observed [39]. Pralnacasan has also been administered to mice during acute DSS-induced colitis, resulting in significant amelioration of disease activity [40].

3. Possible mechanisms for the protective role of ICE inhibition in IBD

3.1. Biological activities of IL-1β and IL-18

Monocytes/macrophages are the best studied source of IL-18 [41]. Most of the information on the production of IL-18 is derived from mice preconditioned with *Propionibacterium acnes* and subsequently challenged with lipopolysaccharide, the original model used to isolate and clone this cytokine [42]. In this particular model, Kupffer cells in the liver are the major producers of IL-18 [42,43]. Differently from what was previously observed for IL-1β [44,45], constitutive gene expression for IL-18 is present in unstimulated, freshly isolated human peripheral blood mononuclear cells as well as murine splenocytes [46] (see also Fig. 1). Constitutive expression of IL-18 mRNA has been observed in many hematopoietic cell lines [47]. The structure of the promoter region of the *IL-18* gene provides insight into these observations. The promoter for *IL-18* does not contain a TATA box, and promoter activity upstream of exon 2 acts constitutively [48]. The additional finding that the 3′ untranslated region of human *IL-18* lacks the AUUUA destabilization sequence is also consistent with these observations [48].

IL-18 acts via an IL-18 receptor complex. The IL-18 receptor complex consists of two non-identical chains: a ligand binding chain termed IL-18α and a non-ligand binding chain termed IL-18β [49–51]. IL-18 does not directly induce IFNγ and other Th1 cytokines [52], but acts together with IL-2 or IL-12 as a costimulant. The synergism between IL-12 and IL-18 and the subsequent effects on the immune system are illustrated in Fig. 2.

IL-1β is not constitutively expressed in healthy subjects [53,54]. Intracellularly, IL-1β is found diffusely in the cytosol rather than localized in the endoplasmic reticulum or Golgi structures [55,56] (Fig. 1). Nearly all microbes and microbial products induce production of IL-1β, but stimulants of nonmicrobial origin can also stimulate transcription [57]. Stimuli such as complement factor 5a, hypoxia, adherence to surfaces, and clotting blood induce the synthesis of large amounts of IL-1β mRNA in monocytic cells without significant translation into IL-1β protein. A second stimulus, such as pro-inflammatory cytokines including IL-1β itself, activates translational mechanisms resulting in translation of the IL-1β mRNA. A review on the biological actions of IL-1β can be found in Ref. [57].

3.2. Cleavage of IL-1β and IL-18 by ICE

Posttranslational, enzymatic processing is a critical step in the modulation of the activity of several cytokines. The

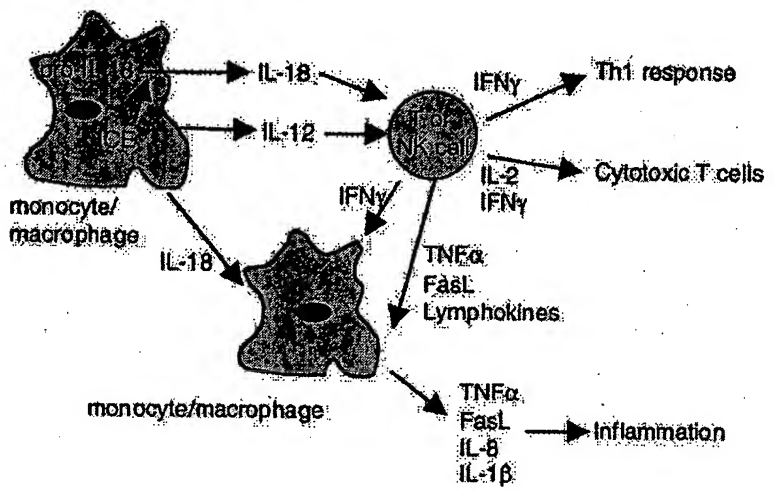

Fig. 2. Biological activities of IL-18 and cell activation. Starting at the upper left, monocytic/macrophagic cells use ICE to cleave inactive pro-IL-18 into active IL-18, which is then secreted from the cell. The same cells also release IL-12 by an ICE-independent mechanism. The combination of IL-18 and IL-12 causes T lymphocytes and natural killer (NK) cells to produce IFNγ, which (a) acts on macrophages to increase ICE expression and further activates the macrophage, and (b) activates $CD4^+$ T lymphocytes as part of the Th1 response. The IL-18/IL-12-stimulated T cell or NK cell releases several lymphokines, TNFα, and/or Fas ligand (FasL). In turn, these cytokines stimulate macrophages to release TNFα, FasL, IL-8, and IL-1β, which result in increased inflammation. The IL-18/IL-12-stimulated T lymphocyte or NK cell also releases IFNγ and IL-2, which result in the generation of cytotoxic T cells.

genes for some cytokines do not encode for a typical signal sequence common to the vast number of secretory, structural, and membrane-bound proteins. IL-1β and IL-18 are examples of cytokines lacking a leader peptide [7,57,58]. These cytokines gain access to the extracellular environment via secretory mechanisms that are linked to their processing. The relationship between the unprocessed IL-1β and IL-18 and the mature form of both cytokines is essential to identify the pleiotropic functions of these molecules. Both pro-IL-1β and pro-IL-18 are biologically inactive [57].

Following synthesis, pro-IL-1β remains primarily cytosolic until it is cleaved and transported out of the cell (Fig. 1). Release of mature IL-1β is often linked to the cleavage at the aspartic acid–alanine (amino acid 116–117) site by ICE [59]. If pro-IL-1β is released into the extracellular space, for example, following cell death or necrosis, there are several extracellular proteases that can cleave pro-IL-1β into an active cytokine [60–63].

3.3. IL-1β and intestinal inflammation

The role of IL-1β in intestinal inflammation depends upon both the up-regulation of IL-1β production and the level of its naturally occurring inhibitor, the IL-1 receptor antagonist (IL-1Ra). Indeed, there is evidence that the balance of IL-1 and IL-1Ra may affect disease outcome. For example, mice deficient in IL-1Ra develop spontaneous rheumatoid arthritis and lethal arteritis [64,65]. Administration of IL-1Ra reduces disease severity in several models of intestinal inflammation [57], including decreased immune complex-induced colitis severity in rabbits [66]. A review of the role of IL-1β in intestinal inflammation has been provided in Ref. [67].

3.4. IL-18 and intestinal inflammation

Several studies provide strong direct and indirect evidence for a significant role of IL-18 in intestinal inflammation. Nakamura and colleagues confirmed *in vivo* the synergistic effect of IL-12 and IL-18, originally described *in vitro* [68], by concomitant injection of both cytokines or either one alone [69]. While mice injected with IL-18 alone did not present with macroscopic changes, IL-12-injected mice showed significant weight loss and colitis. However, the combined administration of both cytokines resulted in severe colitis and high mortality. Chikano and colleagues [70] confirmed this *in vivo* synergism and showed, in addition, that the intestinal inflammation occurs in an IFNγ-dependent but TNFα-, NO- and Fas ligand-independent manner. Consistent with an increased Th1 response in Crohn's disease, several groups could independently demonstrate a significant up-regulation of IL-18 expression in the inflamed lesions of the intestine, mostly localized to macrophages and epithelial cells [71,72]. Interestingly, no increase in IL-18 expression could be observed in inflamed lesions from patients with ulcerative colitis, which is partly characterized by an increase of Th2 cytokines [72]. While increased IL-18 expression is providing first evidence for a possible role in disease, in order to prove that IL-18 participates in the inflammatory process of intestinal inflammation it has to be demonstrated that blockade of IL-18 results in amelioration of disease severity.

In the recent literature, four studies using different animal models of colitis and different ways of IL-18 blockade approach this question. An overview is provided in Table 1, and the studies are summarized in the subsequent paragraphs. Our group examined the role of IL-18

Table 1
Blockade of IL-18 in experimental colitis in mice

Animal model of colitis	IL-18 blocking strategy	Study results	References
Dextran sulfate sodium	Anti-IL-18 antiserum	• IL-18 expression localized to intestinal epithelial cells • Colitis aggravation accompanied by IL-18 increase • Histological amelioration of colitis by anti-IL-18 treatment • Significant decrease in IFNγ, IL-18, and TNFα in the colon after anti-IL-18 treatment	[73]
Trinitrobenzene sulfonic acid	Anti-IL-18 antibody	• IL-18 expression localized to macrophages • Anti-Mac1-saporin antibody as well as neutralizing antibody against IL-18 resulted in a dramatic histological attenuation of colitis • TNBS cannot induce significant colitis in the IL-18 knockout mice • Reduction in IFNγ in IL-18 knockout, anti-IL-18 or anti-Mac1-saporin-treated mice	[77]
Trinitrobenzene sulfonic acid	IL-18 binding protein	• Significantly less histological signs of inflammation after hIL-18BPa treatment • Significant reduction in TNFα, IL-1β, and IL-6, but no decrease in IFNγ, IL-10, and IL-4 in colon homogenates	[78]
$CD62L^+$ $CD4^+$ transfer model	Local administration of adenovirus expressing IL-18 antisense mRNA	• IL-18 expression localized to intestinal epithelial and some mononuclear cells in the lamina propria • Significant decrease in inflammation after treatment, as evaluated histologically and endoscopically • Attenuation of IL-18 synthesis in lamina propria mononuclear cells after treatment	[81]

blockade in the model of DSS-induced colitis [73]. In this model, colitis is induced chemically and is associated with the up-regulation of pro-inflammatory cytokines [74]. Colitis can be induced in *severe combined immunodeficiency* mice by DSS administration and hence is not T cell-dependent [75]. However, during the course of DSS-induced colitis, T cells become activated at the inflammation site and participate in the inflammatory process [76]. In this model, an anti-IL-18 antiserum was administered to achieve IL-18 blockade. IL-18 expression could be localized to the epithelial cells and was reduced significantly in anti-IL-18-treated mice. Blockade of IL-18 was accompanied by a significant reduction in the release of other pro-inflammatory cytokines. In addition, histological signs of inflammation were decreased significantly by blockade with anti-IL-18.

Kanai and colleagues [77] investigated the model of TNBS-induced colitis and focused in particular on the role of IL-18 produced by macrophages. It is hypothesized that the ethanol used as a vehicle in the rectal administration of TNBS disrupts the mucosal epithelial barrier, enabling this hapten to bind covalently to proteins of colonic epithelial cells and modify cell surface proteins. Fragments of these altered cells can be taken up by macrophages and dendritic cells for presentation to T cells as antigens, resulting in a Th1-dominated colitis. Earlier studies showed that administration of neutralizing IL-12 antibodies and thereby blockade of the Th1 pathway is protective [3]. In mice treated with an anti-IL-18 antibody as well as in *IL-18* knockout mice, TNBS was unable to induce significant colitis. An increase in IL-18 synthesis in this model could be localized to the macrophages. In fact, administration of an antibody conjugated to the ribosome-inactivating protein saporin directed against macrophages also resulted in protection against TNBS-induced colitis.

Ten Hove and colleagues [78] also examined the model of TNBS-induced colitis; however, they applied a different strategy to neutralize IL-18. In this study, IL-18BP, a naturally occurring IL-18 antagonist, was administered [79]. The human *IL-18BP* gene encodes for four different isoforms (a–d) generated by alternative mRNA splicing [80]. These isoforms vary in their ability to bind IL-18; only human IL-18BP isoform a (hIL-18BPa) and isoform c have a neutralizing ability, and recombinant hIL-18BPa neutralizes murine IL-18. In this study, mice were treated with recombinant hIL-18BPa during the course of TNBS-induced colitis. Blockade of IL-18 by the recombinant hIL-18BPa resulted in a reduced clinical score accompanied by reduction of TNFα, IL-6, and IL-1β in the colon homogenate, while IFNγ, IL-10, and IL-4 remained unchanged.

Finally, Wirtz and colleagues [81] neutralized IL-18 by local administration of an adenovirus expressing IL-18 antisense mRNA in the T cell-dependent transfer model of colitis. This model is based on the transfer of $CD62L^+$ $CD4^+$ T cells in *severe combined immunodeficiency* mice resulting, after 6–12 weeks, in chronic colitis, which is histologically similar to Crohn's disease in humans [82]. In this study, a significant reduction in IL-18 by adenovirus infected mice expressing IL-18 antisense mRNA was accompanied by a decrease in endoscopically and histologically evaluated inflammation as well as in IFNγ production.

Interestingly, when comparing these four studies, the *IL-18* knockout mice as well as the administration of the neutralizing antibody, the antiserum, or the adenovirus expressing IL-18 antisense mRNA seem to result in a more

dramatic amelioration than the administration of recombinant hIL-18BPa. In particular, in the *IL-18* knockout mice, as well as in the studies with the neutralizing anti-IL-18 antibody/antiserum or after local administration of the adenovirus expressing IL-18 antisense mRNA, a significant reduction in colonic IFNγ concentrations was observed. However, no suppression could be measured in mice after recombinant hIL-18BPa treatment [73,77,78,81]. These differences are of significance and may point out that, although in theory all strategies described aim at the neutralization of IL-18, the IL-18BP might exert additional biological functions that are currently unknown and require further investigations in the future.

The pathogenesis of inflammatory bowel diseases remains elusive. However, results from these experimental models, in combination with the descriptive data from patients with Crohn's disease, suggest an important function of IL-18.

4. Conclusions

The studies in the chronic and acute DSS model suggest that the ICE may contribute to IBD. However, further investigations on the effect of ICE inhibitors in other animal models of experimental colitis are necessary to evaluate whether a similar efficacy can be achieved when compared with the results obtained from single IL-18 or IL-1β blockade. In addition, an IL-1β- and IL-18-independent, yet unknown, mechanism might contribute to the anti-inflammatory function of ICE blockade. Compared to currently used strategies to suppress specific cytokines, which mostly implicate antibody therapy, the possibility of having an orally available drug whose half-life can be easily controlled is highly intriguing.

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References

[1] Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology 1998;115:182–205.
[2] Sandborn WJ, Hanauer SB. Antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results, and safety. Inflamm Bowel Dis 1999;5:119–33.
[3] Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. J Exp Med 1995;182:1281–90.
[4] Simpson SJ, Shah S, Comiskey M, de Jong YP, Wang B, Mizoguchi E, Bhan AK, Terhorst C. T cell-mediated pathology in two models of experimental colitis depends predominantly on the interleukin 12/signal transducer and activator of transcription (Stat)-4 pathway, but is not conditional on interferon γ expression by T cells. J Exp Med 1998;187:1225–34.
[5] Fantuzzi G, Dinarello CA. Interleukin-18 and interleukin-1β: two cytokine substrates for ICE (caspase-1). J Clin Immunol 1999;19:1–11.
[6] Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. Human ICE/CED-3 protease nomenclature. Cell 1996;87:171.
[7] Gu Y, Kuida K, Tsutsui H, Ku G, Hsiao K, Fleming MA, Hayashi N, Higashino K, Okamura H, Nakanishi K, Kurimoto M, Tanimoto T, Flavell RA, Sato V, Harding MW, Livingston DJ, Su MS-S. Activation of interferon-γ inducing factor mediated by interleukin-1β converting enzyme. Science 1997;275:206–9.
[8] Wilson KP, Black J-AF, Thomson JA, Kim EE, Griffith JP, Navia MA, Murcko MA, Chambers SP, Aldape RA, Raybuck SA, Livingston DJ. Structure and mechanism of interleukin-1β converting enzyme. Nature 1994;370:270–5.
[9] Schönbeck U, Mach F, Bonnefoy J-Y, Loppnow H, Flad H-D, Libby P. Ligation of CD40 activates interleukin 1β-converting enzyme (caspase-1) activity in vascular smooth muscle and endothelial cells and promotes elaboration of active interleukin 1β. J Biol Chem 1997;272:19569–74.
[10] McAlindon ME, Hawkey CJ, Mahida YR. Expression of interleukin 1β and interleukin 1β converting enzyme by intestinal macrophages in health and inflammatory bowel disease. Gut 1998;42:214–9.
[11] Young JL, Sukhova GK, Foster D, Kisiel W, Libby P, Schonbeck U. The serpin proteinase inhibitor 9 is an endogenous inhibitor of interleukin 1beta-converting enzyme (caspase-1) activity in human vascular smooth muscle cells. J Exp Med 2000;191:1535–44.
[12] Dimmeler S, Haendeler J, Nehls M, Zeiher AM. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1β-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. J Exp Med 1997;185:601–7.
[13] Li J, Billiar TR, Talanian RV, Kim YM. Nitric oxide reversibly inhibits seven members of the caspase family via S-nitrosylation. Biochem Biophys Res Commun 1997;240:419–24.
[14] Kim Y-M, Talanian RV, Li J, Billiar TR. Nitric oxide prevents IL-1β and IFN-γ-inducing factor (IL-18) release from macrophages by inhibiting caspase-1 (IL-1β-converting enzyme). J Immunol 1998;161:4122–8.
[15] Fantuzzi G, Puren AJ, Harding MW, Livingston DJ, Dinarello CA. Interleukin-18 regulation of interferon γ production and cell proliferation as shown in interleukin-1β-converting enzyme (caspase-1)-deficient mice. Blood 1998;91:2118–25.
[16] Netea MG, Fantuzzi G, Kullberg BJ, Stuyt RJL, Pulido EJ, McIntyre Jr RC, Joosten LAB, Van der Meer JWM, Dinarello CA. Neutralization of IL-18 reduces neutrophil tissue accumulation and protects mice against lethal *Escherichia coli* and *Salmonella typhimurium* endotoxemia. J Immunol 2000;164:2644–9.
[17] Norman J, Yang J, Fink G, Carter G, Ku G, Denham W, Livingston D. Severity and mortality of experimental pancreatitis are dependent on interleukin-1 converting enzyme (ICE). J Interferon Cytokine Res 1997;17:113–8.
[18] Rau B, Paszkowski A, Lillich S, Baumgart K, Möller P, Beger HG. Differential effects of caspase-1/interleukin-1β-converting enzyme on acinar cell necrosis and apoptosis in severe acute experimental pancreatitis. Lab Invest 2001;81:1001–13.
[19] Paszkowski AS, Rau B, Mayer JM, Möller P, Beger HG. Therapeutic application of caspase 1/interleukin-1β-converting enzyme inhibitor decreases the death rate in severe acute experimental pancreatitis. Ann Surg 2002;235:68–76.
[20] Ku G, Ford P, Raybuck SA, Harding MW, Randle JCR. Selective interleukin-1 converting enzyme (ICE/Caspase-1) inhibition with pralnacasan (HMR3480/VX-740) reduces inflammation and joint destruction in murine type II collagen-induced arthritis (CIA). Arthritis Rheum 2001;44:S241.

[21] Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martin J, Carrascal T, Walsh P, Reznikov LL, Kim S-H, Novick D, Rubinstein M, Dinarello CA. IL-18 regulates IL-1β-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Natl Acad Sci USA 2000;97:734–9.

[22] Mathan MM, Mathan VI. Morphology of rectal mucosa of patients with shigellosis. Rev Infect Dis 1991;13(Suppl 4):S314–8.

[23] Islam D, Veress B, Bardhan PK, Lindberg AA, Christensson B. In situ characterization of inflammatory responses in the rectal mucosae of patients with shigellosis. Infect Immun 1997;65:739–49.

[24] Zychlinsky A, Prevost MC, Sansonetti PJ. *Shigella flexneri* induces apoptosis in infected macrophages. Nature 1992;358:167–9.

[25] Sansonetti PJ, Arondel J, Cavaillon J-M, Huerre M. Role of interleukin-1 in the pathogenesis of experimental shigellosis. J Clin Invest 1995;96:884–92.

[26] Zychlinsky A, Fitting C, Cavaillon J-M, Sansonetti PJ. Interleukin 1 is released by murine macrophages during apoptosis induced by *Shigella flexneri*. J Clin Invest 1994;94:1328–32.

[27] Zychlinsky A, Thirumalai K, Arondel J, Cantey JR, Aliprantis AO, Sansonetti PJ. In vivo apoptosis in *Shigella flexneri* infections. Infect Immun 1996;64:5357–65.

[28] Hilbi H, Chen Y, Thirumalai K, Zychlinsky A. The interleukin 1β-converting enzyme, caspase 1, is activated during *Shigella flexneri*-induced apoptosis in human monocyte-derived macrophages. Infect Immun 1997;65:5165–70.

[29] Walsh JA. Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality. Rev Infect Dis 1986;8:228–38.

[30] Kanani SR, Knight R. Relapsing amoebic colitis of 12 year's standing exacerbated by corticosteroids. Br Med J 1969;2:613–4.

[31] Li E, Stanley Jr SL. Protozoa. Amebiasis. Gastroenterol Clin North Am 1996;25:471–92.

[32] Tucker PC, Webster PD, Kilpatrick ZM. Amebic colitis mistaken for inflammatory bowel disease. Arch Intern Med 1975;135:681–5.

[33] Zhang Z, Yan L, Wang L, Seydel KB, Li E, Ankri S, Mirelman D, Stanley Jr SL. *Entamoeba histolytica* cysteine proteinases with interleukin-1 beta converting enzyme (ICE) activity cause intestinal inflammation and tissue damage in amoebiasis. Mol Microbiol 2000;37:542–8.

[34] Darwin KH, Miller VL. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clin Microbiol Rev 1999;12:405–28.

[35] Monack DM, Detweiler CS, Falkow S. Salmonella pathogenicity island 2-dependent macrophage death is mediated in part by the host cysteine protease caspase-1. Cell Microbiol 2001;3:825–37.

[36] Monack DM, Hersh D, Ghori N, Bouley D, Zychlinsky A, Falkow S. Salmonella exploits caspase-1 to colonize Peyer's patches in a murine typhoid model. J Exp Med 2000;192:249–58.

[37] Monack DM, Navarre WW, Falkow S. Salmonella-induced macrophage death: the role of caspase-1 in death and inflammation. Microbes Infect 2001;3:1201–12.

[38] Siegmund B, Lehr H-A, Fantuzzi G, Dinarello CA. IL-1β-converting enzyme (caspase-1) in intestinal inflammation. Proc Natl Acad Sci USA 2001;98:13249–54.

[39] Randle JC, Harding MW, Ku G, Schönharting M, Kurrle R. ICE/Caspase-1 inhibitors as novel anti-inflammatory drugs. Expert Opin Investig Drugs 2001;10:1207–9.

[40] Bauer C, Loher F, Schmall K, Hallwachs R, Loehr C, Siegmund B, Dauer M, Lehr HA, Schönharting M, Endres S, Eigler A. *In vivo* efficacy of the Il-1β converting enzyme inhibitor pralnacasan in DSS-induced murine colitis. Gastroenterology 2002;122(Suppl. 1):T971.

[41] Dinarello CA. IL-18: a TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol 1999;103:11–24.

[42] Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S, Kurimoto M. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature 1995;378:88–91.

[43] Tsutsui H, Nakanishi K, Matsui K, Higashino K, Okamura H, Miyazawa Y, Kaneda K. IFN-γ-inducing factor up-regulates Fas ligand-mediated cytotoxic activity of murine natural killer cell clones. J Immunol 1996;157:3967–73.

[44] Mileno MD, Margolis NH, Clark BD, Dinarello CA, Burke JF, Gelfand JA. Coagulation of whole blood stimulates interleukin-1β gene expression. J Infect Dis 1995;172:308–11.

[45] Shapiro L, Dinarello CA. Hyperosmotic stress as a stimulant for proinflammatory cytokine production. Exp Cell Res 1997;231:354–62.

[46] Puren AJ, Fantuzzi G, Dinarello CA. Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1β are differentially regulated in human blood mononuclear cells and mouse spleen cells. Proc Natl Acad Sci USA 1999;96:2256–61.

[47] Akita K, Ohtsuki T, Nukada Y, Tanimoto T, Namba M, Okura T, Takakura-Yamamoto R, Torigoe K, Gu Y, Su MS, Fujii M, Satoh-Itoh M, Yamamoto K, Kohno K, Ikeda M, Kurimoto M. Involvement of caspase-1 and caspase-3 in the production and processing of mature human interleukin 18 in monocytic THP.1 cells. J Biol Chem 1997;272:26595–603.

[48] Tone M, Thompson SAJ, Tone Y, Fairchild PJ, Waldmann H. Regulation of IL-18 (IFN-γ-inducing factor) gene expression. J Immunol 1997;159:6156–63.

[49] Born TL, Thomassen E, Bird TA, Sims JE. Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. J Biol Chem 1998;273:29445–50.

[50] Parnet P, Garka KE, Bonnert TP, Dower SK, Sims JE. IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologues T1/ST2 and IL-1R AcP. J Biol Chem 1996;271:3967–70.

[51] Torigoe K, Ushio S, Okura T, Kobayashi S, Taniai M, Kunikata T, Murakami T, Sanou O, Kojima H, Fujii M, Ohta T, Ikeda M, Ikegami H, Kurimoto M. Purification and characterization of the human interleukin-18 receptor. J Biol Chem 1997;272:25737–42.

[52] Ushio S, Namba M, Okura T, Hattori K, Nukada Y, Akita K, Tanabe F, Konishi K, Micallef M, Fujii M, Torigoe K, Tanimoto T, Fukuda S, Ikeda M, Okamura H, Kurimoto M. Cloning of the cDNA for human IFN-γ-inducing factor, expression in *Escherichia coli*, and studies on the biologic activities of the protein. J Immunol 1996;156:4274–9.

[53] Fenton MJ, Clark BD, Collins KL, Webb AC, Rich A, Auron PE. Transcriptional regulation of the human prointerleukin 1β gene. J Immunol 1987;138:3972–9.

[54] Fenton MJ, Vermeulen MW, Clark BD, Webb AC, Auron PE. Human pro-IL-1β gene expression in monocytic cells is regulated by two distinct pathways. J Immunol 1988;140:2267–73.

[55] Rubartelli A, Cozzolino F, Talio M, Sitia R. A novel secretory pathway for interleukin-1β, a protein lacking a signal sequence. EMBO J 1990;9:1503–10.

[56] Stevenson FT, Torrano F, Locksley RM, Lovett DH. Interleukin 1: the patterns of translation and intracellular distribution support alternative secretory mechanisms. J Cell Physiol 1992;152:223–31.

[57] Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996;87:2095–147.

[58] Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW, Kornfeld H. Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem 1998;273:1144–9.

[59] Black RA, Kronheim SR, Cantrell M, Deeley MC, March CJ, Prickett KS, Wignall J, Conlon PJ, Cosman D, Hopp TP, Mochizuki DY. Generation of biologically active interleukin-1β by proteolytic cleavage of the inactive precursor. J Biol Chem 1988;263:9437–42.

[60] Coeshott C, Ohnemus C, Pilyavskaya A, Ross S, Wieczorek M, Kroona H, Leimer AH, Cheronis J. Converting enzyme-independent release of tumor necrosis factor α and IL-1β from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. Proc Natl Acad Sci USA 1999;96:6261–6.

[61] Dinarello CA, Cannon JG, Mier JW, Bernheim HA, LoPreste G, Lynn DL, Love RN, Webb AC, Auron PE, Reuben RC, Rich A, Wolff SM,

Putney SD. Multiple biological activities of human recombinant interleukin 1. J Clin Invest 1986;77:1734–9.

[62] Mizutani H, Black R, Kupper TS. Human keratinocytes produce but do not process pro-interleukin-1 (IL-1) beta. Different strategies of IL-1 production and processing in monocytes and keratinocytes. J Clin Invest 1991;87:1066–71.

[63] Mizutani H, Schechter N, Lazarus G, Black RA, Kupper TS. Rapid and specific conversion of precursor interleukin 1β (IL-1β) to an active IL-1 species by human mast cell chymase. J Exp Med 1991;174:821–5.

[64] Horai R, Saijo S, Tanioka H, Nakae S, Sudo K, Okahara A, Ikuse T, Asano M, Iwakura Y. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. J Exp Med 2000;191:313–20.

[65] Nicklin MJ, Hughes DE, Barton JL, Ure JM, Duff GW. Arterial inflammation in mice lacking the interleukin 1 receptor antagonist gene. J Exp Med 2000;191:303–12.

[66] Cominelli F, Nast CC, Duchini A, Lee M. Recombinant interleukin-1 receptor antagonist blocks the proinflammatory activity of endogenous interleukin-1 in rabbit immune colitis. Gastroenterology 1992; 103:65–71.

[67] Cominelli F, Pizarro TT. Interleukin-1 and interleukin-1 receptor antagonist in inflammatory bowel disease. Aliment Pharmacol Ther 1996;10:49–53.

[68] Barbulescu K, Becker C, Schlaak JF, Schmitt E, Meyer zum Büschenfelde K-H, Neurath MF. Cutting edge: IL-12 and IL-18 differentially regulate the transcriptional activity of the human IFN-γ promoter in primary $CD4^+$ T lymphocytes. J Immunol 1998;160:3642–7.

[69] Nakamura S, Otani T, Ijiri Y, Motoda R, Kurimoto M, Orita K. IFN-γ-dependent and -independent mechanisms in adverse effects caused by concomitant administration of IL-18 and IL-12. J Immunol 2000; 164:3330–6.

[70] Chikano S, Sawada K, Shimoyama T, Kashiwamura S-I, Sugihara A, Sekikawa K, Terada N, Nakanishi K, Okamura H. IL-18 and IL-12 induce intestinal inflammation and fatty liver in mice in an IFN-γ dependent manner. Gut 2000;47:779–86.

[71] Monteleone G, Trapasso F, Parrello T, Biancone L, Stella A, Iuliano R, Luzza F, Fusco A, Pallone F. Bioactive IL-18 expression is up-regulated in Crohn's disease. J Immunol 1999;163:143–7.

[72] Pizarro TT, Michie MH, Bentz M, Woraratanadharm J, Smith Jr MF, Foley E, Moskaluk CA, Bickston SJ, Cominelli F. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. J Immunol 1999;162:6829–35.

[73] Siegmund B, Fantuzzi G, Rieder F, Gamboni-Robertson F, Lehr H-A, Hartmann G, Dinarello CA, Endres S, Eigler A. Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-γ and TNF-α production. Am J Physiol Regul Integr Comp Physiol 2001;281:R1264–73.

[74] Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG, Van Rees EP. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol 1998;114:385–91.

[75] Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. Gastroenterology 1994;107:1643–52.

[76] Saubermann LJ, Beck P, De Jong YP, Pitman RS, Ryan MS, Kim HS, Exley M, Snapper S, Balk SP, Hagen SJ, Kanauchi O, Motoki K, Sakai T, Terhorst C, Koezuka Y, Podolsky DK, Blumberg RS. Activation of natural killer T cells by α-galactosylceramide in the presence of CD1d provides protection against colitis in mice. Gastroenterology 2000; 119:119–28.

[77] Kanai T, Watanabe M, Okazawa A, Sato T, Yamazaki M, Okamoto S, Ishii H, Totsuka T, Iiyama R, Okamoto R, Ikeda M, Kurimoto M, Takeda K, Akira S, Hibi T. Macrophage-derived IL-18-mediated intestinal inflammation in the murine model of Crohn's disease. Gastroenterology 2001;121:875–88.

[78] Ten Hove T, Corbaz A, Amitai H, Aloni S, Belzer I, Graber P, Drillenburg P, van Deventer SJH, Chvatchko Y, Te Velde AA. Blockade of endogenous IL-18 ameliorates TNBS-induced colitis by decreasing local TNF-α production in mice. Gastroenterology 2001;121:1372–9.

[79] Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immunity 1999;10:127–36.

[80] Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M, Dinarello CA. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc Natl Acad Sci USA 2000;97:1190–5.

[81] Wirtz S, Becker C, Blumberg R, Galle PR, Neurath MF. Treatment of T cell-dependent experimental colitis in SCID mice by local administration of an adenovirus expressing IL-18 antisense mRNA. J Immunol 2002;168:411–20.

[82] Atreya R, Mudter J, Finotto S, Müllberg J, Jostock T, Wirtz S, Schütz M, Bartsch B, Holtmann M, Becker C, Strand D, Czaja J, Schlaak JF, Lehr HA, Autschbach F, Schürmann G, Nishimoto N, Yoshizaki K, Ito H, Kishimoto T, Galle PR, Rose-John S, Neurath MF. Blockade of interleukin 6 *trans* signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in Crohn disease and experimental colitis in vivo. Nat Med 2000;6:583–8.